Developing a novel protocol for testing decontamination of TiO2 dental implant surfaces- an in vitro experimental study

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Introduction

Endo-osseous titanium dental implants are coated with an oxide layer which promotes adhesion of biomolecules, cellular binding, and hence osseointegration. Contamination of the implant surface will reduce the surface tension which will affect the adhesion capacity in a negative manner [1]. Peri-implantitis has been defined as "an inflammatory process affecting the tissues around an osseointegrated dental implant in function, resulting in loss of supporting bone"[2]. A review by Roos-Jansaker and co-workers [1] states that the prevalence of peri-implantitis reported in the literature is in the range 1-19%.

It seems to be of vast importance to decontaminate the affected titanium dental implant surface during surgical intervention with the aim to accomplish re-osseointegration.
A wide range of decontaminating techniques has been suggested in the literature, such as carbondioxide laser irradiation, tetracycline solution, citric acid and hydrogen peroxide. Various methods of mechanical debridement of titanium implants have been suggested such as carbon fiber curettes, plastic curettes and titanium curettes. A common concern for all commercially available instruments is the difficulties getting sufficient access in between the treads of the contaminated fixtures. We suggest that utilization of a titanium brush will lead to significantly better result.
To the best of our knowledge, no studies evaluating decontamination of a titanium implant, using a titanium brush, have been presented in the literature.

Our first aim was to develop a novel methodology for evaluation of surface decontamination of dental implants. Secondly we had the aim to compare surface decontamination with either a novel titanium brush or titanium curettes.

Materials and methods

We have developed a novel methodology for testing of surface decontamination of dental implants. We also utilized this methodology to test decontamination of smooth surfaced commercially pure titanium implants using either a titanium brush or a titanium curette. We fabricated 30 half cylinder threaded titanium implants (Fig 1.). The implants were rinsed according to a well established protocol ([3] All implants were incubated in 50 ml of foetal calf serum (FCS)for 24 hours in 37◦ C to accomplish a protein saturated surface. They was thereafter stored in a +4◦ C fridge until experimental decontamination. Prior to experimental decontamination the implants was rinsed in Phosphate Buffered saline (PBS, Dulbeco) for 10 seconds and thereafter submerged in a solution of 10% foetal calf serum and 90% PBS.

Fourier Transformation Infrared Spectroscopy, FT IR (Spectrum 400, Perkin Elmer, Norway) with drift mode was used to analyse the organic contaminations on ten implants. The resolution of the DR FT-IR was set to 16,m and average scan to five. Spectra were recorded in the wavelength range from 4000 to 450 cm^{-1}. The peak area and height was quantified by Spectrum software (Spectrum v 2.1, Perkin Elmer, Oslo, Norway) for the four most abundant peaks. Twenty implants were taken out of the solution one by one. (1)Ten implants were debrided with a titanium brush for 60 seconds. The brush rotated with 1000 Rpm. A new brush was used for each implant, Ten implants were debrided with a titanium curettes for 60 seconds (Fig 3). During all debridements, with both curettes and brush, irrigation with profuse amounts of saline was performed . Ten implants were left untreated as control implants.

The curettes were sharpened using a Arkansas stone for 30 seconds between each time of experimental debridement. All experimental debridement was performed by a board certified periodontist.

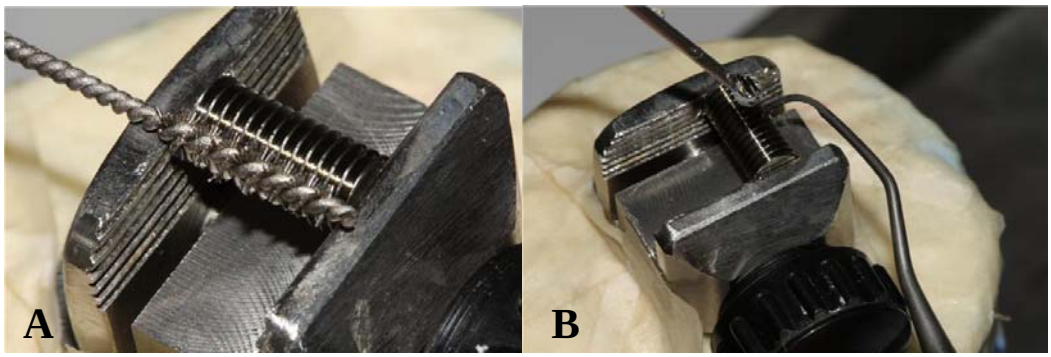

Fig1. A. Titanium Brush, B. Titanium curette

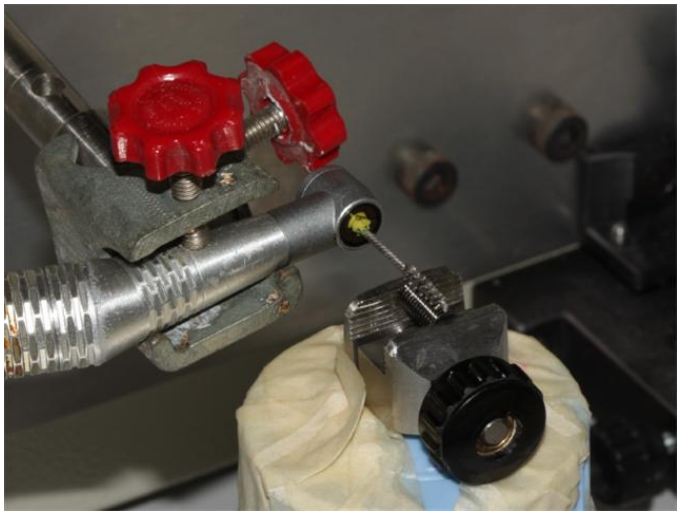

Fig 2. The titanium brush was placed in a reciprocal handpiece and the lever was standardized. The force used on the titanium curette was not standardized.

After experimental debridement the implants were critical point dried and thereafter analysed using spectrophotometry. The whole procedure was done in one seanse: first decontaminated, then critical point dried and then analysed.

After decontamination and analysis the implants where put in a solution of PBS and stored in the fridge (+4*C) until further quantification of proteins on the surface.

The quantification of proteins on the surface was done by using a protein assay (Bio-Rad Laboratories Hercules, CA, USA). The implants were put in separate chambers and 100 µl of an extraction buffer containing 2ml of 10mM tris- HCl, pH 7.5, 7.5 ml 150mM NaCl, 2 ml 1% Triton y-100, 2 ml 1 % sodiumdodecylsulfat, 400 mikroliter 1 mM EDTA and dest. water to a final volume of 200 ml were added.. Testtubes where marked and put on ice. The coins including all fluid where transferred to marked testtubes. Each tube was vortexed for 30 sec and then placed in -20°C fridge until quantification of proteins were done using the protein essay.

All the samples were taken out of the fridge and shaken (vortexed) for 5 seconds. An aliqoute of 25 µl where taken out of each sample and placed in new eppendorph tubes. A BCA solution where made of 50 parts of reagent A and 1 part of reagent B (50:1, reagent A:B). 200 µl of WR were added to each sample and shaken (vortex) for 30 seconds. A standardized curve for the concentration of Albumin where made. The samples were analyzed using ELISA.

Statistics

The data from the protein analysis was compared using the Mann-Whitney U test. The FT-IR results was compared using Dunn`s test. The alpha value was 0.005

Results

There was a statistically significant difference between the remaining proteins after decontamination with the titanium brush compared to the titanium-curette ($p<0.05$). The

tianiumcurette removed significantly more protein than the titaniumbrush.

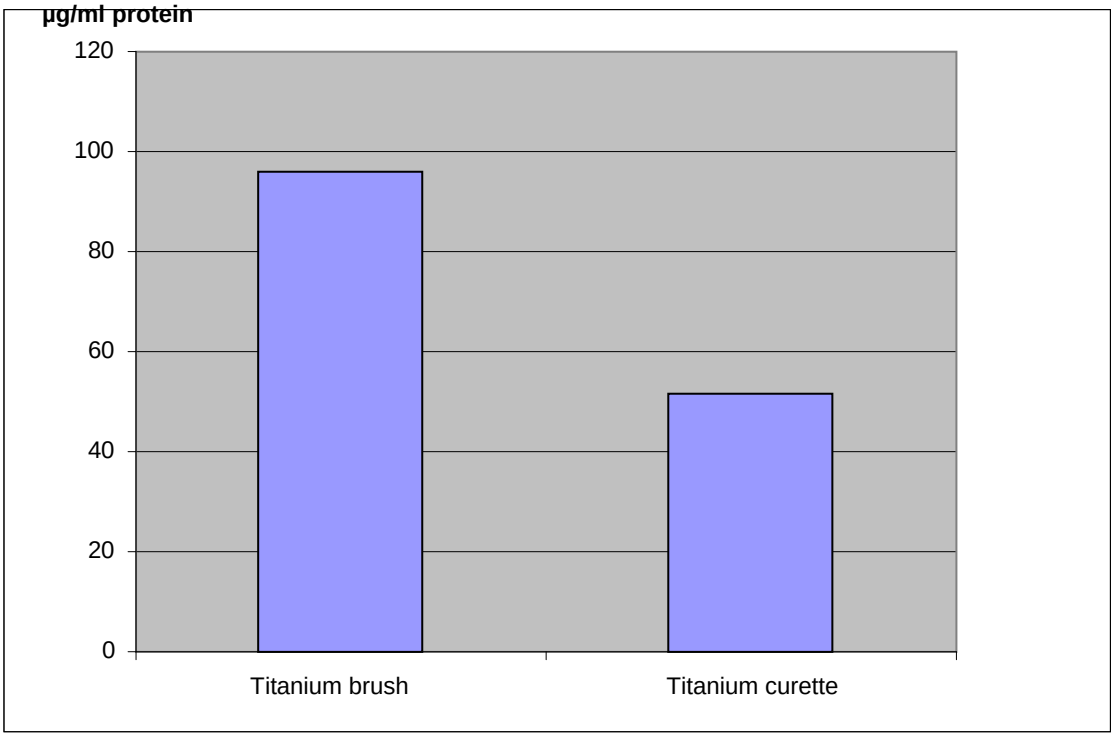

Figure3. Quantification of remaining protein after cleaning with a titanium brush and a Titanium curette

The FT-IR analysis showed four distinctive peaks at 1651, 1540, 1300 and 846 cm-1 (Fig 2). The two first were identified as amide bonds. The absorption bands in the amide I (1650 cm−1) and is mainly due to combination of C=O and C-N stretchings of amide groups. Amide II (1540 cm−1) is mainly due to in-plane C-N stretching and N-H bending of amide groups. The latter two groups were identified as bond between C-H. There was a significant decline the total peak height area for these two peaks (Fig. 3). The titanium curette also left

less organic material with C-H on the surface than both control and the titanium brush (Dunn's test, $p<0.05$).

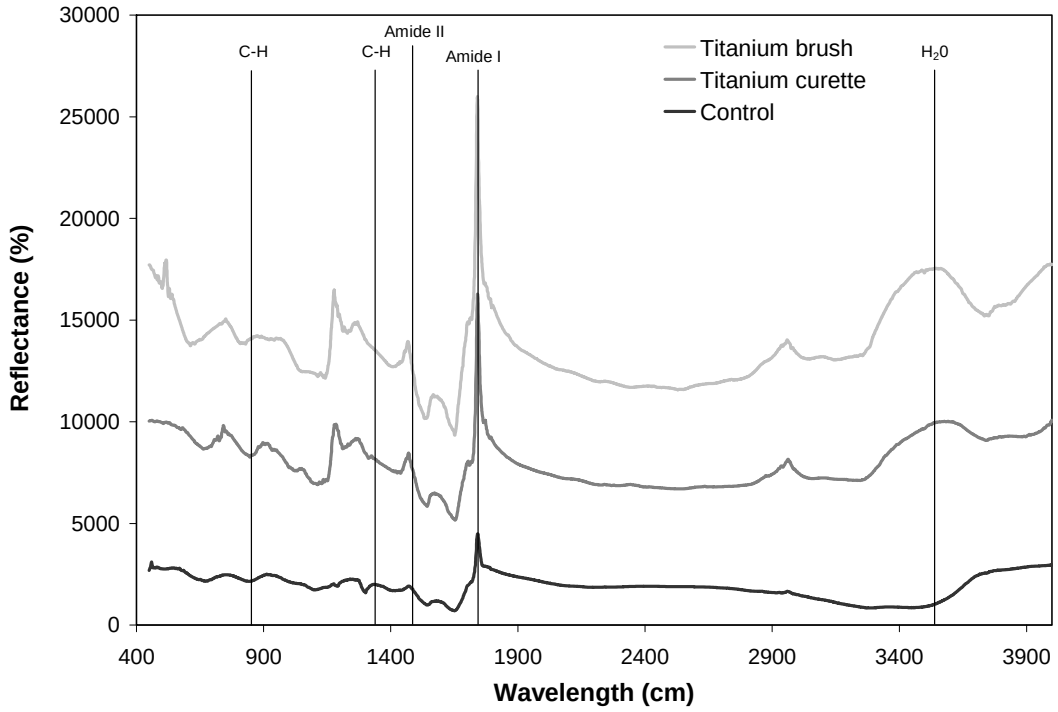

Figure 4: Three selected spectra from the DR FT-IR analysis. Four major peaks (amide I, amide II and C-H) were closely investigated

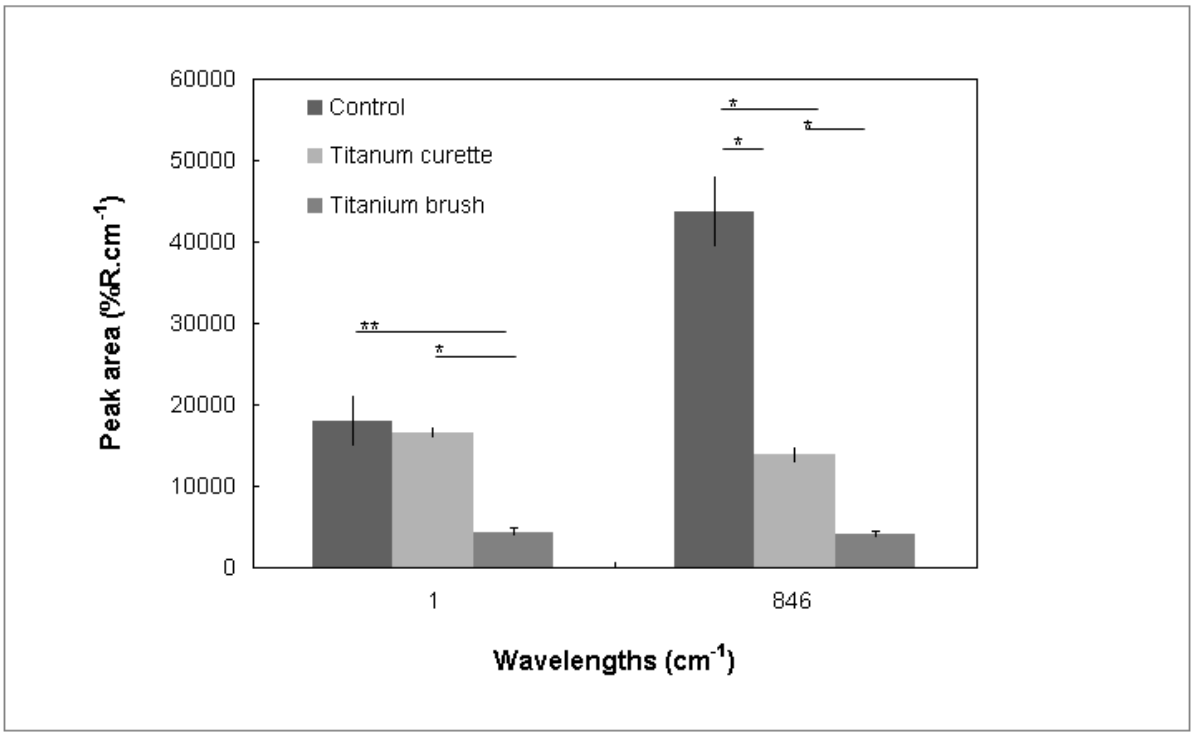

Figure 5 .Mean area of the reflectance from the 846 and 1300 cm-1 peaks and standard error of the mean as error bars (*p<0.05, **p<0.01). A significant decrease was detected for the titanium brush compared to curette and control for both wavenumbers.

Discussion

We had the aim at (1) developing a new methodology for testing dental implants surface decontamination and (2) compare two type of methodologies for surface decontamination namely utilization of a titanium brush versus titanium curettes.

Finding an appropriate protocol for surface decontamination of dental implants was of outmost importance. Close to 10 million dental implants are being placed each year.

Recent studies are reporting that a substantial number of these implants will be affected by

peri-implantitis [4, 5]. As of today very limited evidence exists for one specific treatment strategy for peri-implantitis, but there seems to be a general understanding that implants affected by peri-implantitis needs to be decontaminated if re-osseointegration shall occur [6]. A number of surface decontamination strategies have been reported in the literature but so far with inconclusive evidence.

It was important to find a methodology which will decontaminate dental implants but at the same time leave the original surface structure intact, not obstructing the potential for re-osseointegration. Using carbonfiber curettes has been reported to leave remnants of carbonfiber on the implant surface. Similarly the utilization of dental lasers has been reported to burnish the surface which may have a negative effect on the potential for the implant to re-osseointegrate.

After developing the methodology we only tested the two methods once. The methodology at this stage should not be considered to give a rigid outcome and needs further calibration.

It was difficult to standardize the decontamination methodology. The reciprocal brush and the handpiece with the titanium brush were placed in a weighted lever in an attempt to standardize the force used with the titanium brush. It was very difficult to maintain contact between the brush and the titanium-surface which certainly may have had an effect on the outcome of the test. Mechanical debridement with the titanium curette was utilized using regular hand force. This force was with no hesitation much higher than what was used with the brush which certainly has a major impact on the results. It will be of utmost importance to develop a methodology for standardization of the force used.

Generally, Amide I band has a composite band profile, consisting of several spectral components related to the different secondary structures [7]. However, spectral overlaps

between Amide I band and strong ν2 absorption band of water at approximately 1650 cm−1 has been reported. It is likely that this had occurred here. Rinsing the samples with D2O prior to measurement, may have solved the problem with water contaminations. Therefore, more focus was laid on the strong adsorption bonds at 1300 and 846 cm-1.

It will also be of utmost importance to evaluate the above results with the perspective of hazardous scratchings on the implant surface. A cleaning method should fulfill the important criteria of not causing damage to the implant surface. This needs to be further evaluated. We suggest further and repeated testing of these two methodologies for surface decontamination before any conclusions can be drawn.

Conclusion

Our preliminary results seem to demonstrate that a titanium curette is more efficient than a titanium brush in removing proteins on a titanium dental implant. This study does not evaluate damage on the implant surface after the various decontamination methods. This will be an important parameter for further testing. It will also be of outmost importance to standardize the forces that are used. Further testing will be necessary before any conclusions can be drawn.

References

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Attachments 1.

Cleaning protocol:

Astratech AB (cleaning of implants):

Stage 1

1.Wash implants with 70% ethanol

2.Implants in deionized water 40 *C and ultrasound for 5 min

3.Implants in NaOH 40% and waterbath 40*C for 10 min

4.Implants in deionized, ultrasound 5 min

5.pH control, by washing implants (D. Water) until pH becomes 6.

6.Implants can stay in Ethanol 70 % or directly to next stage.

Stage 2

1.Implants I deionized water 50*C and ultrasound for 5 min

2. Implants in HN03 50%, 50 *C waterbath for 10 min

3.Implants in D. water, ultrasound for 5 min

4. pH control, by washing implants (D.water) until pH become 6

5. Implants in ethanol 70 %